REMARKS

Claims 28, 29, 31, 33-35, 37-41, 43 and 44 have been amended. Claims 30, 36, 45 and 46 have been canceled. Thus, claims 1-29, 31-35 and 37-44 are now presented for examination. Support for the claim amendments may be found in the original and canceled claims, and throughout the specification, particularly at pages 3-14. Reconsideration and withdrawal of the present rejections in view of the amendments and comments presented herein are respectfully requested.

Rejections under 35 U.S.C. § 102(b)

Zhang et al.

Claims 28-43 were rejected under 35 U.S.C. § 102(b) as being anticipated by Zhang et al. (*Proc. Natl. Acad. Sci. USA* **94**:4504-4509, 1997).

Zhang *et al.* discloses the evolution of a beta-galactosidase to a fucosidase using misincorporation mutagenesis and gene shuffling, wherein a chromogenic substrate was used to screen mutants on plate assays. In order for a claim to be anticipated by a reference, each element of the claim must be found within the reference. In the present case, Zhang *et al.* does not teach or suggest a method as recited in the amended claims which allows the detection and recovery of all mutants which are still functional (but not necessarily better than the wild type by any arbitrary test) which can be used as starting material for additional rounds of mutagenesis. In addition, Zhang *et al.* neither teaches nor suggests a method which allows the accumulation of multiple mutations within the mutant genes during each round of mutagenesis as recited in the amended claims. Thus, since Zhang et al. does not disclose the above-referenced features of the presently claimed invention, the claims cannot be anticipated by this reference.

Rai et al.

Claims 28-31 and 33-36 were rejected under 35 U.S.C. § 102(b) as being anticipated by Rai et al. (WO99/20768).

Rai *et al.* discloses a method of using misincorporation mutagenesis to produce novel amylases. Like Zhang et al,, Rai *et al.* does not teach or suggest a method as recited in the

amended claims which allows the detection and recovery of all mutants which are still functional (but not necessarily better than the wild type by any arbitrary test) which can be used as starting material for additional rounds of mutagenesis.. In addition, Rai *et al.* does not teach step (d) (sorting host bacteria having the detectable characteristic by flow cytometry, wherein host bacteria are sorted without selecting for an altered or defined level of enzyme activity compared with a corresponding wild type enzyme; and obtaining a pool of mutated genes encoding functional enzymes from the bacteria in step (d) and repeating steps (a) to (d) to form a library of bacteria containing a plurality of mutant genes expressing a functional enzyme). Thus, in contrast to the method recited in the present claims, Rai *et al.* does not teach or suggest a method which allows the accumulation of multiple mutations within the mutant genes during each round of mutagenesis. Thus, since Rai et al. does not disclose the above-referenced features of the presently claimed invention, the claims cannot be anticipated by this reference.

Moreover, the presently claimed method places no selective bottleneck on the population of mutants. The only selection criterion is that each mutant enzyme still possesses sufficient enzymatic activity to produce a level of enzyme product that will allow a bacterium (containing the enzyme) to be differentiated from bacterium containing no active enzyme, and that the bacteria can be detected by flow cytometry. The methods disclosed in the Zhang et al. and Rai et al. references apply a selective bottle-neck after mutagenesis, in which only a small subset of improved mutants are selected to go into the next round of mutagenesis. By removing this selective bottleneck, the presently claimed mutagenesis method allows for the accumulation of neutral, mildly negative (not inactivating) and positive mutations into the population of mutant genes. This allows rapid generation of functional mutant genes containing multiple amino acid changes. The accumulation of multiple mutations increase the likelihood of finding useful mutations, or mutations which change an enzyme's characteristics.

In view of the comments presented above, Applicants respectfully request reconsideration and withdrawal of the rejections under 35 U.S.C. § 102(b)

Rejection under 35 U.S.C. § 103(a)

Claim 44 was rejected under 35 U.S.C. § 103(a) as being unpatentable over Zhang et al. in view of Rice et al. (PNAS, 89:5467-5471, 1992). The Examiner alleges that it would have been obvious to apply the flow cytometry techniques of Rice to the mutagenesis method of

Zhang for the predictable results of enabling rapid sorting of the host microorganisms as suggested by Rice.

As described above, the pending claims are not anticipated (or rendered obvious by) Zhang et al. Establishing *prima facie* obviousness requires a showing that each claim element is taught or suggested by the prior art. *See* In re Royka, 490 F.2d 981, 180 USPQ 580. (CCPA 1974). Rice *et al.* teaches a general method for screening randomly mutagenized expression libraries in host cells by flow cytometry (FACS), but does not remedy the deficiencies in the teaching of Zhang *et al.* Thus, one of ordinary skill in the art would not combine the teaching of Zhang *et al.* and Rice *et al.* to develop a method which allows the detection and recovery of all mutants which are still functional (but not necessarily better than the wild type by any arbitrary test) which can be used as starting material for additional rounds of mutagenesis to arrive at the presently claimed invention

In view of the comments presented above, Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a).

No Disclaimers or Disavowals

Although the present communication may include alterations to the application or claims, or characterizations of claim scope or referenced art, the Applicants are not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. The Applicants reserve the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not reasonably infer that the Applicants have made any disclaimers or disavowals of any subject matter supported by the present application.

CONCLUSION

Applicants submit that all claims are in condition for allowance. However, if minor matters remain, the Examiner is invited to contact the undersigned at the telephone number provided below. Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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Dated: 7/8/08

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